NCI ETI Branch
Flow Cytometry Core Laboratory

Protocol for labeling cells with CFDA-SE (carboxyfluorescein diacetate succinimidyl ester).

Reagents. This protocol is has been found useful for labeling both primary cells and cell lines with the fluorescent probe CFDA-SE (carboxyfluorescein diacetate succinimidyl ester). This probe is often referred to incorrectly in the literature as "CFSE" – it should NOT be confused with carboxyfluorescein succinimidyl ester (the real CFSE), which is not the diacetate form and is not cell-permeable. The correct reagent can be obtained from Molecular Probes, catalog number C-1157.

Stock and storage. We prepare CFDA-SE at a stock concentration 1000-fold higher than the final usage concentration (for example, 2 mM if the final concentration is 2 uM) in dry DMSO. Aliquot into single-usage vials and store over desiccant at –20^{o}C. CFDA-SE will hydrolyze quickly at room temperature in the presence of water, and much more slowly at –20^{o}C under desiccating conditions. Aliquoted stocks should be used for no more than 2 months. If your cells show decreased labeling with the same stock of CFDA-SE, hydrolysis is the likely cause.

Labeling concentration and conditions. Cells are usually labeled at a final CFDA-SE concentration of 0.5 to 5 uM. The literature reports concentrations ranging from 0.2 to 10 uM and even higher. For best results, do a titration and find the lowest concentration of CFDA-SE that will give effective cell labeling – this will vary from cell type to cell type, and also with the application. CFDA-SE labeling is somewhat toxic and can induce growth arrest and apoptosis in some cell types – therefore, it is important to find the lowest acceptable labeling concentration and *check the viability* after labeling. As a rough guide, 0.5 to 2 uM is usually enough for *in vitro* experiments – cell tracking and generational analysis in transplanted cells may require 2 to 5 uM. Incubation time is usually from 5 to 10 minutes – again, titrate to find the minimal effective conditions. We usually label in PBS or HBSS containing 0.1% BSA. All post-labeling washes should be carried out in complete media (such as RPMI with 10% FBS) – your intended tissue culture media is ideal. The high protein concentration inactivates unreacted CFDA-SE.

1. Suspend your cells at in PBS or HBSS containing 0.1 BSA%. Cell concentrations can range widely from 1 x 10^6 cells/ml (for *in vitro* experiments) up to 5 x 10^7 cells/ml (for adoptive transfer). The cells should be in single cell suspension – if necessary, filter them through nylon mesh immediately prior to labeling. Total reaction volumes should not exceed 4 ml in a 15 ml tube, so prepare cell suspensions at no greater than 2 ml each.

2. Prepare a solution of CFDA-SE from your DMSO stock in PBS/0.1% BSA at 2X the final labeling concentration. For example, if you are labeling at 5 uM, prepare a 10 uM solution. Prepare a volume of CFDA-SE equal to your cell volume above (no more than 2 ml per labeling reaction).
3. Add an equal volume of CFDA-SE solution to your cell suspension. Mix gently and incubate for 5 to 10 minutes at 37^{o}C.
4. Immediately fill the labeling tube to the top with the tissue culture media intended for culture (such as RPMI/10% FBS) and centrifuge. Wash the cells three times with tissue culture media at room temperature. To reduce the amount of unbound CFDA-SE in cells, we usually incubate the cells at 37^{o}C for 5 minutes after the second wash and prior to the third. This allows free unreacted CFDA-SE to diffuse out of the cells and be removed in the final wash.

This protocol was prepared by the Telford Lab for the NCI Medicine Branch and its friends.
11-18-00